REMARKS

Reconsideration and continuing examination of the above-identified application is respectfully requested in view of the amendments above and the discussion that follows.

Withdrawal of the rejection of claims 1-46 under 35 USC 112, first paragraph is noted with appreciation. Claims 1, 25, and 47 have been amended. Claims 1-47 are in the case and are currently under examination.

I. The Amendments

Claims 1, 25, and 47 have been amended to more properly comply with grammatical outline conventions. There is no new matter, merely the re-labelling of the steps of these claims.

II. The Action

A. Claim Rejections

1. Rejections under 35 USC §112, First Paragraph

a. It is noted with appreciation that the rejection of claims 1-46 under 35 USC §112, first paragraph for containing new matter is withdrawn.

b. Claims 1-47 were rejected under 35 USC §112, First Paragraph, because allegedly, the specification while being enabling for a HBc chimer of SEQ ID NO:1, does not reasonably provide enablement for a HBc chimer containing up to about 5 percent substituted amino acid residues in the HBc SEQ ID NO:1.

It is first noted that there is no stated reason for asserting that the claims do not enable a HBc chimer containing up to about 5 percent substituted amino acid residues in the HBc

SEQ ID NO:1. The Court in *In re Marzocchi* & Horton, 169 USPQ 367, 369-370 (CCPA 1971) ruled upon the propriety of a similar breadth rejection in a chemical application. The Court held that the

> [o]nly relevant concern on the Patent Office under these circumstances should be over the truth of any such assertion [of efficacy]. [169 USPQ at 369; emphasis in the original].

The Court went on to hold:

> It is incumbent upon the Patent Office, whenever a rejection on this basis [doubt as to enablement] is made, to explain <u>why</u> it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate statement (Emphasis in the original.)

The *Marzocchi* requirement of an explanation of "why" truth or accuracy of the disclosure is doubted has not been complied with. The Action has therefore not complied with the legal requirements, and this rejection should therefore be withdrawn.

Still further, the text at pages 73-75 discusses various substitutions and several more are shown in the sequence of Fig. 1 for the various human viral strains. Thus, the text at page 75 notes "[m]ore preferably, about 9 residues are different from the *ayw* sequence (SEQ ID NO: 1) at residue positions 2-183, . . ." and that "[d]ifferences in shorter sequences, e.g., 2-149, or 2-156, or 2-163 are proportional to

those discussed before based on percentage." The Examiner's attention is invited to the four human HBc viral sequences shown in Fig. 1 and included as SEQ ID NOs:1-4. Examination of those sequences will show seven possible substitutions that can be made in the *ayw* sequence of SEQ ID NO:1 from positions 2-156. In addition, another six residue substitutions are discussed in the text at pages 73-73; i.e., DI by EL at positions 2-3, L to R at positions 7 and 97 and C to S at positions 48 and 107. It is submitted that those substitutions are more than a sufficient number to constitute "about 5 percent" and again, this rejection should be withdrawn.

2. Rejections under Doctrine of Obviousness-Type Double Patenting

The Action asserted that claims 1-46 were rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over (1) claims 1-78 of 09/930,915; (2) Claims 1-53 of 10/787,734; (3) Claims 98-109 of 10/805,913; (4) Claims 79-115 of 10/806,006; and (5) Claims 47-58 of 11/508,655.

The Examiner's comments about obviousness-type double patenting are noted. However, inasmuch as no claims of any of the applications cited in the Action are noted to be allowable, let alone allowed, it is still believed to be premature to deal with a terminal disclaimer.

It is respectfully requested that this rejection now be withdrawn.

3. Rejections under 35 USC §103(a)

a. Pumpens in view of Zlotnick and Zheng

Claims 1-6, 8-14, 16-28, 30-42 and 46 were again rejected under 35 USC §103(a) as being unpatentable over Pumpens in view of Zlotnick and Zheng. This rejection is again respectfully traversed.

The Action first discusses the alleged contribution of the Zlotnick manuscript. The premise that Zlotnick teaches that C-terminal cysteine can stabilize an HBc chimer molecule as recited in the claims here cannot be agreed with. This premise is inconsistent with the statements and data provided therein by Zlotnick.

For example, Zlotnick explicitly states: "[p]urified Cp*149 and Cp*150 assemble into capsids under the same conditions as other constructs, with or without DTT. These capsids were *indistinguishable* (emphasis added) by negative staining electron microscopy and sedimentation on sucrose gradients." (See page 9558, column 1, paragraph 1, Results and Discussion section.) As a second example, Zlotnick reports: "[a]t a resolution of ≈20Å, the outer surface of the Au11-labeled [monomaleimidyl-undecagold-labeled] Cp*150 capsid is *indistinguishable* (emphasis added) from those of unlabeled Cp147 and Cp183 capsids, (cf. Fig. 4 Top)." (See page 9558, column 2, paragraph 1.) These facts would lead one skilled in the art to conclude that C-terminal cysteines are not important for HBcΔ capsid formation or stability.

The Zheng manuscript echoes this conclusion. For example, Zheng states: "[e]ach of the cysteines of HBcAg has been eliminated both singly and in combination. All the proteins were shown to have very similar physical and immunochemical properties. All assemble into essentially

identical core particle structures. Therefore, disulfide bonds are not essential for core particle formation." (see page 9422, Abstract).

It is believed that these statements alone are strong evidence to refute the Action's proposal that Zlotnick teaches that C-terminal cysteine can stabilize HBcΔ particles. However, there are other matters that also support that point.

One matter is that the Action relies heavily on Figure 2 of Zlotnick in support of its arguments. It is respectfully noted that the polyacrylamide gel shown therein depicts disulfide bonded *dimers* not HBcΔ core protein capsid particles. Those capsids are the entities recited in the claims to have enhanced stability.

Examination of Fig. 2a of Zlotnick reveals that the greatest mass there shown for reduced and non-reduced protein corresponded to a little more than that of the 31 kDa molecular weight standard. That molecular weight is that expected for a dimer of two strands of 150 residues (150 residues X 109 average molecular wt/residue = 16.35 kDa). The reported results in that figure therefore relate only to dimers and monomers.

The present claims recite stability of the particles assembled from those monomers and dimers. As such, a disclosure concerning the stability or lack thereof of dimers or monomers neither teaches nor suggests anything of relevance to the claimed subject matter whether taken alone or with any other disclosure. It is not logical to conclude that large molecular weight, structurally folded, dense capsid molecules that contain 90 or 120 dimers would behave identically to their constituent dimers. One of skill in the art would recognize this.

It is not logical to conclude that large molecular weight, structurally folded, dense capsid molecules that contain

90 or 120 dimers would behave identically to their constituent dimers. One of skill in the art would recognize this.

Nothing in Zlotnick has shown that capsids behave like dimers. In fact, reading an above-quoted statement with the Action's premise, Zlotnick teaches that capsids do not behave like dimers. For example, Zlotnick states: "[a]ssembled capsids of reduced Cp*150 did not react with Au11. Apparently, the C-terminal cysteine is inaccessible. Accordingly, Cp*150 was labeled with Au11 at neutral pH and low ionic strength, where the sample is mainly free dimers." (See page 9558, column 1, paragraph 3.) Therefore, it is improper to conclude that capsids behave like dimers as was done in the Action in reference to Figure 2 of Zlotnick.

Importantly, it further appears that in Zlotnick's Figure 2, two crucial controls are lacking. One missing control is a sequence of the truncated 150-mer with the C-terminal cysteine replaced by an alternative amino acid. Without this crucial control, one of skill in the art would reasonably conclude that the polymerization of Cp*150 was due to the increase in length of the peptide from 149 amino acids to 150 amino acids. This is a perfectly plausible conclusion given Zlotnick's quoted statement "[p]urified Cp*149 and Cp*150 assemble into capsids under the same conditions as other constructs, with or without DTT. These capsids [i.e., particles] were *indistinguishable* (emphasis added) . . ." In fact, this is the *only* difference between Cp*149 and Cp*150. Zlotnick did not show otherwise.

This conclusion is even more plausible given the fact the addition of gold at the C-terminus also promotes aggregation. Zlotnick states: "[t]hus it appears that modification of Cp*150 with Au11 promotes polymerization. The

Au11 has a single reactive maleimide group and cannot crosslink proteins." (See page 9558, column 1, paragraph 3.) Also, Zlotnick states: "[o]ther observations imply that the C termini also may influence assembly in more subtle ways. For instance binding Au11 to Cp*150 induces assembly, though Au11 cannot crosslink subunits, nor, because of its organic shell, coordinate C-terminal cysteines." (See page 9540, column 2, paragraph 1.)

In that same paragraph, while relating to the gold-labeled mutants, Zlotnick further goes on to hypothesize that changes in the molecular surface near the C-terminus may stimulate the assembly process. Therefore, one of skill in the art would reasonably conclude from these statements that Zlotnick teaches that dimer formation is induced by merely increasing length of the mutant protein, since aggregation also occurs in the absence of free cysteine at the C-terminus of the protein.

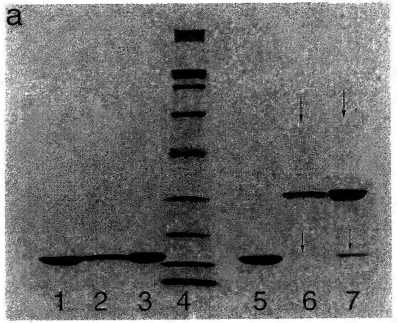
The second control that is lacking in Zlotnick is that of Cp*150 with the internal cysteines at positions 48, 61 and 107 in place. Without this second crucial control, one of skill in the art could not conclude that any alleged increase in capsid stability of Figure 2B was due to terminal cysteines as proposed in the Action. The alleged increase in dimerization shown in Figure 2A could also be due to the removal of those amino acids, which might have profound effects on the folding and stability of the molecule as is shown in the present application. Zlotnick does not provide any data nor does he discuss any proteins with internal cysteines.

The only C-terminal mutant protein that Zlotnick shows in the polyacrylamide gel and size exclusion chromatography of Figure 2 does not contain any internal cysteines, unlike the

mutants of the present application. One of ordinary skill in the art would recognize that the presence or absence of these moieties would undoubtedly change the structure, folding and intramolecular binding of the mutant protein. Zlotnick does not take into account this critical feature in the design of his experiments, and neither has the Action in its assertions about the relevance of the Zlotnick disclosures.

The present inventors have properly accounted for the presence and absence of internal cysteines and it is a notable difference between the present application and the Zlotnick, Zheng, and Pumpens manuscripts. Paragraph [0043] of the present application states: "[t]hus self-assembled particles are typically more stable than are particles formed from otherwise identical HBc chimer molecules that contain both cysteine residues at positions 48 and 107 after storage at 37°C in a 20mM sodium phosphate buffer at pH 6.8 for a time period of one month. Thus, the absence of one, or more preferably, both cysteines at residue positions 48 and 107 can enhance the storage ability of a particle that is otherwise stabilized by the presence of an N- or C-terminal cysteine or both. The replacement of one or both of those cysteine residues can also enhance the expression of the chimer molecule particles." Also see paragraph [0527] of the present application, which states: "[s]ignificantly, all particles (except non-C-terminally stabilized controls) that were incubated at 37 degrees C were entirely disulfide bonded after a period of 7 days. Of particular note was the fact that the C-terminally stabilized C48S/C107S chimer appeared to be entirely disulfide bonded at day zero, whereas its C48/C107 counterpart was not and did not reach the same level of cross-linking achieved by the C48S/C107S chimers during the period of study."

Still further, upon careful scrutiny of Zlotnick's cited gel (Fig. 2a, shown below with arrows added by the undersigned), one can see in lane 7 that



there is a band at the lower edge of the gel near the spot of 14.4 kDa standard that clearly indicates that some of the Cp*150 sample has either failed to associate into a dimer or has readily dissociated at pH 9.5. There is a similar band at pH 7.5. A recently obtained sharper, color copy of the Zlotnick paper is attached for the Examiner's convenience.

The gel of Zlotnick's Fig. 2a (above) also shows two protein bands at a molecular mass between 45 kDa and 66 kDa that are also delineated by counsel's upper arrows. No evidence of the presence of capsids is shown in the gel. However, those heavier bands indicate some sort of instability of the products formed.

The size exclusion chromatography graph in Fig. 2b is not informative. No standards were shown. Without some standard to measure by, one cannot reliably state what this graph shows.

What can be said, however, is that that depicted study also lacked appropriate controls. Indeed, the study shown in Figure 2B might have related to relative stability of the capsids. However, inasmuch as there were no data for either another 150 residue HBc with no cysteines at all, a 150 residue HBC with the three internal cysteines at positions 48, 61 and 107, or a 150 residue HBC with the three internal cysteines at positions 48, 61 and 107 as well as a C-terminal Cys, or a chimer containing an insert at the N-terminus, the C-terminus or in the loop along with appropriate cysteines, one cannot apply the illustrated results of that study to predict anything about the claimed subject matter.

It is therefore submitted that both Zlotnick and Zheng teach that C-terminal cysteines are not important for HBcΔ capsid formation.

The Action next discusses the alleged contributions of the Zheng manuscript. The assertion that Zheng teaches that Cys48 and Cys107 are not essential for native core particle formation is agreed with. The basis for this assertion is the Abstract of Zheng on page 9422, as mentioned above. It must be emphasized that Zheng teaches that *no* disulfide bonds are necessary for particle formation. That is the same teaching Zlotnick made in the first quoted portion above taken from page 9558, column 1, paragraph 1. This result for particle formation is in contrast to the particle stability exhibited by the truncated chimer proteins of the present invention that contain at least two cysteine moieties per molecule (at position 61 and at/near one or both of the N- and C-termini).

Importantly, Zlotnick also teaches that neither of the cysteines at positions 48, 61, and 107 are vital for particle formation. His mutant where those cysteines were all replaced

by alanines still formed particles identical to the native 183-mer particle. (see page 9558, column 2, paragraph 1).

As for Pumpens, this article was not mentioned in the discussion of claim rejections in the last Action although the rejection is maintained. The statement from the previous Action that Pumpens does not teach replacing one or both cysteines at position 48 and 107 by another residue and adding a C-terminal cysteine is agreed with.

Therefore, given the knowledge that (1) terminal cysteines are not important for capsid formation by Zlotnick and Zheng and Pumpens; (2) the disulfide bonds of Cys 48, 61 and 107 are not necessary for particle formation by Zheng and Zlotnick and Pumpens, one of skill in the art would not be motivated by the combination of these teachings to produce the chimeric HBc proteins of the present application. In fact, one would be dissuaded from doing so in that the data cited therein would indicate that such chimers would be unsuccessful and ineffective vaccine carriers. This basis for rejection should be withdrawn.

b. Page and Birkett both in view of Zheng

Also, claims 1-6, 8-28, 30-46 again were rejected under 35 USC §103(a) as being unpatentable over Page and Birkett both in view of Zheng. This rejection is again respectfully traversed.

It is asserted that it would not have been obvious to make the molecules of the present invention in light of the combination of teachings of Page, Birkett, and Zheng. None of the disclosures teach that the removal of cysteines at positions 48 and 107 of the chimer molecules would enhance the stability of the subsequently formed capsid, and none of them teaches or

suggests that the addition of a terminal cysteine to such a capsid results in added stability as well.

Support for this conclusion comes from the Action, which states that Page does not explicitly teach incorporating a heterologous linker residue for a conjugated epitope present in the HBc immunodominant loop between amino acid residues 76 and 85 and does not teach replacing one or both cysteine residues at positions 48 and 107 by another residue.

The Action then states that Birkett teaches the incorporation of a heterologous linker residue for a conjugated epitope present in the immunodominant loop of HBc. Notably, Birkett does not teach replacing one or both cysteines at positions 48 and 107, nor does Birkett address the added stability of such capsids with C-terminal cysteines.

Zheng teaches that no cysteines are important for capsid particle formation, as described in the previous argument above. Zheng is silent on the stabilizing effects of cysteines at any location.

Therefore, the present invention as a whole was not *prima facie* obvious to one of ordinary skill in the art based on the combination of these three manuscripts as alleged in the Action. It is respectfully requested that this rejection be withdrawn.

B. Summary

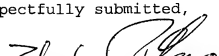
Claims 1, 25 and 47 have been amended. Each of the bases for rejection has been dealt with and overcome or otherwise made moot.

It is therefore believed that this application is in condition for allowance of all of the pending claims. An early notice to that effect is earnestly solicited.

No further fee or petition is believed to be necessary. However, should any further fee be needed, please charge our Deposit Account No. 23-0920, and deem this paper to be the required petition.

The Examiner is requested to phone the undersigned should any questions arise that can be dealt with over the phone to expedite this prosecution.

Respectfully submitted,

By 
Edward P. Gamson, Reg. No. 29,381

WELSH & KATZ, LTD.
120 South Riverside Plaza, 22nd Floor
Chicago, Illinois 60606
Phone (312) 655-1500
Fax No. (312) 655-1501

Enclosures
Petition and fee
Zlotnick paper in color